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Intraparenchymal Transplantation

GOPAL D. DAS

Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

I. Introduction

Neural transplantation, in principle, involves transplantation of embryonic neural tissue in the central nervous system or in the ventricular cavities or subdurally on the surface of the host brain. Placement of neural tissue at the time of transplantation, whether inside the host brain or outside it, helps define intraparenchymal versus extraparenchymal transplantation. However, following transplantation, as the neural tissue grows, it may remain inside the host brain and be parenchymally integrated with it and become an intraparenchymal transplant, or it may not show any significant growth and remain isolated and unintegrated in a cavity within the host brain and thus become an extraparenchymal transplant. Thus, conceptually, it is important to distinguish procedure of transplantation from the ultimate fate of the transplant. Intraparenchymal transplantation refers to the placement of a transplant inside the host brain at the time of surgery, and intraparenchymal transplant refers to the final picture of the transplant after it has grown and differentiated (Das, 1983a).

Before discussing intraparenchymal transplantation it is important to stress some conditions essential for successful transplantation, these are: viability of the neural tissue; retention of the transplant at the site of transplantation; and minimum amount of pathological reaction at the site of transplantation. Generally these three aspects are not considered at all, but

it is important to understand them in order to comprehend the nature of successful transplantation. A viable tissue obtained from embryonic brain is one that after transplantation still shows all the characteristics of a healthy and survivable tissue. The cells in such a transplant appear normal and they may show some degree of proliferation after transplantation. In contrast to this, the non-viable tissues appear necrotic as soon as they are transplanted. There are many conditions that can make a viable tissue non-viable. The most important condition contributing to non-viability is drying up of the tissue when exposed to air during its manipulation from the embryonic brain. If the dissection of tissue is not performed carefully the tissue that is exposed to air immediately becomes necrotic and remains non-viable. Other conditions, such as using non-sterile Ringer's solution or non-sterile procedures of dissection and preparation of tissues, also contribute to their non-viability. The second factor, namely retention of the transplant at the site of transplantation, is a very important one. A transplant that has not been retained in place, and that oozes out from the host brain, does not yield an intraparenchymal transplant. The third factor, namely pathological reaction caused at the site of transplantation by the very technique of injection or insertion of the tissue, significantly affects the survival and growth of the transplant. All the techniques of transplantation are invasive in nature. The very act of injection of neural tissues amounts to an invasive trauma to the host brain. One should make every possible effort to keep pathological reaction to a minimum. If attention is not paid to this extensive damage is caused to the host brain, with the pathological reaction involving extensive hemorrhage, necrosis of cells in the host brain, edema, ischemia, extravasation of blood cells, and extensive gliosis. These conditions do not provide an optimum environment for the initial retention and survival of the transplant, and its subsequent growth and differentiation. Transplants under such conditions invariably become necrotic and die away. Those that survive are very small, undifferentiated, and remain unattached to the host brain. These three conditions of transplantation, in addition to others, are extremely important. If they are not adequately controlled the very survival, growth, differentiation, and integration of transplants may be affected. These three conditions, if uncontrolled and unaccounted, have the potential of contributing to artifacts of techniques and observations.

From a comprehensive viewpoint intraparenchymal transplantation can be defined as injection or deposition of embryonic neural tissue within the host brain in such a manner that it is completely or maximally apposed to the host brain parenchyma at the time of transplantation. This is in opposition to placing a tissue in a ventricle or leaving it subdurally on the surface of the host brain where it is separated from the host brain parenchyma by the intervening pia mater or arachnoid and pia mater. Intraparenchymal trans-

plantation can be achieved by various procedures, but broadly speaking there are two main approaches: injecting the neural tissue within the host brain parenchyma; or first preparing a cavity by surgical means to expose the host brain parenchyma and then depositing the transplant in the cavity. Both these methods provide parenchymal apposition between the transplant and the host brain tissue at the time of transplantation, and both provide favorable conditions for anatomical integration between the two. However, these two techniques may have different influences on the growth and connectivity of the transplants as they grow and differentiate (Das, 1983b).

II. Instruments and Technique

Intraparenchymal transplantation, in our studies, involves injection of neural tissues within the host brain with the aid of a syringe. The most important instrument is the syringe with glass needle. The details on preparation and honing of the glass needle have been given in detail in our earlier publications (Das, 1974; Das et al., 1979). A glass tuberculine syringe of a volume of 0.25–0.5 mm is found to be the most suitable. Into this a 3 cm long thin-walled capillary glass tubing, 0.8 mm in outer diameter and 0.6 mm in inner diameter, is inserted. This glass capillary serves as a glass needle. It is sealed with the help of epoxy cement. Following this, the glass capillary is gently polished and honed on a very fine grade sharpening stone in order to obtain a bevel as in the metal needles. Bevel of appropriate angle is extremely important for the insertion and penetration of the needle into the host brain, and injection of the tissue at the desired site. If the needle is not beveled, a blunt flat capillary causes damage to the dura mater and the host parenchyma as it is inserted. The syringe with glass needle is allowed to dry overnight, when it is cleaned with alcohol and sterile deionized water. Further, it is desirable to have at least three or four such syringes readily available before starting on surgery. If one needle is broken then other syringes will be at hand for use. The glass needle can be calibrated to determine the exact volume of neural tissue that is being injected. Calibration of the glass needle is a very simple procedure. A known amount of Ringer's solution is taken with the help of a Hamilton microsyringe. A droplet of 1.0 μl is taken into the glass capillary. Its level is marked with a permanent non-toxin ink. With this one can calibrate the needle in terms of 1, 2 or 3 or more μl (1.0 μl = 1.0/mm^3). With this calibration, when the tissue is taken in the needle one can determine how much tissue is being injected. Further, the calibration also aids in taking small quantities such as 0.5 or 0.25 mm^3 for injection. With experience it is possible to inject small amounts of neural tissues in the host brain very precisely.

The second aspect of technique is preparation of the donor embryos and the transplant. Details on these aspects have been given in our earlier publications (Das, 1974; Das et al., 1979). In essence, one can collect rat embryos of any age from embryonic stages of day 12 to day 23. It is relatively easy to dissect all neural tissues from embryos of higher gestational stages than from those of lower stages. In the case of donor embryos of younger developmental stages it is possible to collect only some, and not all, neural tissues uncontaminated with meningeal membranes. For instance, it is possible to dissect spinal cord and brain stem relatively easily in 12-, 13- and 14-day embryos, but not neocortex. This is due to the fact that differentiation and segregation of meningeal membranes in rostral regions lags behind those in the caudal regions of neuraxis. Thus, the ability to collect embryos of lower developmental stages does not necessarily guarantee ability to collect neural tissues without contamination of undifferentiated meningeal membranes and mesenchymal tissues closely attached to the embryonic brain. Further, embryonic neural tissues from embryos of different gestational stages may differ in their cellular composition. Neural tissues may be composed of neuroepithelial cells, or neuroblasts, or a mixture of the two in varying proportions. Depending upon the cellular composition different transplants will show different magnitude of growth. For instance, neocortical tissues from 15-day rat embryos, which are primarily composed of the neuroepithelial cells, show extensive growth. Whereas the same neocortical tissue from 23-day rat embryos, which is mainly composed of neuroblasts, shows very little growth. Thus, the choice of neural tissue depends upon the magnitude of growth of the transplant desired by the investigator. In other words, it is essential to bear in mind that not all neural tissues grow equally large. Finally, it is important to stress the fact that dissection of embryonic neural tissues must be performed when the tissue is fully submerged in Ringer's solution. If the tissue is exposed to air, even for a few seconds, it becomes nonviable. Very little is gained by transplanting non-viable neural tissue, for it does not yield any positive results.

The third aspect of transplantation involves preparation ot the host animals. This largely depends upon the site of the host brain selected by the investigator. If neural tissue has to be transplanted in the forebrain, invariably one must prepare the animals appropriately, and drill a hole in the cranium in order to insert the needle and reach the desired structure. Similarly, if neural tissues have to be injected in the cerebellum one can approach it either through the occipital bone or through the cisterna magna. However, the spinal cord poses totally different problems. It is very difficult to transplant neural tissues through small holes in the intervertebral discs. Our experience has shown that if the tissues are transplanted into the spinal cord through small holes such transplants invariably ooze out, occupy subdural

space and become extraparenchymal. As extraparenchymal transplants they may survive for a few weeks or months before they are completely necrotized. The problems of transplantation in spinal cord have been discussed elsewhere and they will not be dealt with here (Das, 1983b, c). Age of the host animals has an important bearing on their preparation for transplantation. In general, neonatal host animals are easier to prepare than the adult host animals.

Following the above described steps of preparation of neural transplants and preparation of the host animals, the final step involves injection of the neural tissue inside the host brain. For this the neural tissue of the desired volume is taken in the needle, it is gently inserted into the host brain deep inside to reach the desired structure, and the tissue is slowly and gently injected. After the injection of neural tissue the needle is drawn out very slowly. If it is drawn out rapidly the transplant oozes out and settles subdurally. If injection is done carefully, the transplant is retained at the site of its deposition. In such a case there is no need to insert extraneous materials such as gelfoam into the site of transplantation.

The technique described above pertains to transplantation of neural tissues in a normal brain. However, transplantation of neural tissues in a traumatized brain, where a large lesion or cavity has been made, poses totally different problems. In such a case a large surface of the host brain has to be exposed. The site of injury must be cleaned and bleeding must be stopped. Only then can one attempt to transplant neural tissue. In such a case it is important to bear in mind that the neural tissue should be of such growth characteristics that after it has grown it fills the cavity. If the tissue is of low growth potential it may not fill the cavity, and may remain isolated in the pathological environment of the host brain.

III. Advantages

(1) The main advantage of the technique described above on intraparenchymal transplantation is that the transplant at the time of its injection has its entire surface apposed to the host brain parenchyma. This allows for the most important requirement, namely, as the transplant grows and expands it achieves parenchymal attachment on all its sides, thereby establishing interface with the host brain completely. In such preparations, unless some flaw related to contamination of the tissue or excessive bleeding inside the host brain is introduced, the entire transplant is completely integrated with the host brain. There is generally no patch of separation between the transplant and the host brain. This is of utmost importance if it is required that the transplant becomes an integral part of the host brain and survives for the life of the animal.

(2) This method provides standard conditions of obtaining transplants of relatively identical anatomical characteristics in all the animals. Reproducibility of preparations can be achieved with a high degree of success. Quantitative analysis of such transplants for their growth, volume and connectivity can be performed validly and reliably.

(3) This is the only technique that facilitates injection of transplants in the deep structures of the host brain without causing any damage to the surface structures of the brain. Control on pathological reaction is of importance if one wants to have transplants survive, grow, and differentiate normally in the host brain.

(4) In these preparations it is observed that as the transplant grows it simply pushes the host brain outward to occupy the volume of space that it has created by its growth. The amount of host brain tissue lost appears to be commensurate with the volume that the transplant occupies. No excessive amount of host brain tissue is lost or damaged, and no cyst or massive necrotic tissue is left in the host brain.

(5) With this technique retention of transplants at the site of transplantation is very high. The transplants do not ooze out or get slipped to settle subdurally. Because of this the necessity of inserting some foreign substances like gelfoam to hold the transplant in place is eliminated. Placement of the foreign substance often is found to have an adverse effect on the very survival of the transplant.

(6) With the use of the glass needle it is possible to take precise amounts of neural tissues for transplantation. It is possible to use as small an amount as 0.5 or 0.25 mm^3 of tissue, and transplant it successfully without losing it anywhere. Small transplants, with this technique, are seen not only to survive but also to grow and become completely integrated with the host brain.

(7) Further, use of the glass needle permits an inspection of the quality of transplant before it is injected. It is possible to see if there is excessive Ringer's solution or air bubbles in the needle along with the transplant. Injection of excessive Ringer's solution or air bubbles is a potential source of causing edema at the site of transplantation. In contrast to this, if a metallic needle is used it is not possible to see if there are any air bubbles or excessive Ringer's solution along with the transplant.

(8) Finally, the above described technique assumes that the investigator has sufficient skill to inject the transplants slowly and carefully with his hands. If this is not possible, one can overcome this difficulty by employing stereotaxic technique of transplantation. The details on this technique have been described elsewhere in our earlier publication (Das and Ross, 1982).

IV. Disadvantages

As all techniques of transplantation, this technique also has some disadvantages.

(1) This technique is satisfactory only for transplanting in large and deep structures of the host brain, such as basal ganglia, thalamus, hypothalamus, and cerebellum. In these structures the transplants are retained satisfactorily. However, small and thin structures like spinal cord, or superficial structures like cerebral cortex, are not suitable for this technique of transplantation. If this technique is followed, the transplants generally slip or slide down farther away from the site of transplantation and settle subdurally elsewhere following their injection. Such cases of transplantation may lead to erroneous conclusions on the lack of survival of the transplants, when in actuality they have simply not been retained in place satisfactorily. In these conditions one may have to adopt the technique of making a surgical cavity in the cortex or the spinal cord and then injecting the transplant. To ensure that the transplant is retained in the cavity the use of other non-neural substances like gelfoam to hold the transplant in place may become an imperative technical requirement.

(2) Occasionally it is possible that, while injecting the transplant by hand, some tissue may ooze out along the needle track, which may result in an extraparenchymal transplant. This can be controlled by holding the needle in place after injection of the transplant for about 10 sec. The needle should not be allowed to move in any manner. Then the needle should be withdrawn very slowly and gently. This method ensures that the host parenchyma on the surface closes immediately as the needle is withdrawn, and the transplant is retained in its place.

(3) Injecting neural tissues by hand has the flaw of the problem of movement of the needle. If the needle moves too much, an extensive amount of damage may be caused to the host parenchyma. In such a case the transplant is actually deposited in a pathological environment and not in a normal environment. The transplant growing in a pathological environment may achieve totally different characteristics in establishing interface with the host brain than that growing in a normal host brain environment. Therefore it is very important to have good control over the hand. To some extent this problem can be overcome by using the stereotaxic approach of transplantation.

(4) For transplantation in the forebrain or diencephalon or cerebellum, it is necessary to make a small hole in the cranium for inserting the needle. This small hole does not permit a full view of the blood vessels on the surface. It is possible that, as the needle is inserted, some blood vessels on the surface may be damaged and this may cause bleeding. It is not possible to stop bleeding effectively and immediately. Similarly, a penetrating needle may cause dam-

age to the capillary system deep inside the host brain. In such a case there can be extensive hemorrhage at the site of transplantation, and this is undesirable for the very survival of the transplant. However, with experience and practice an investigator may be able to determine the probability of hitting large blood vessels when the needle goes through certain stereotaxic coordinates inside the host brain, and change the coordinates or follow a slightly different approach to avoid extensive bleeding.

V. Conclusions

The above presentation shows that this technique, although valuable for intraparenchymal placement of neural transplants, has some disadvantages as well as advantages. Like any other technique it will not be applicable to all conditions of transplantation. For other conditions of transplantation, as required by the nature of the experiment, one may have to resort to placing transplants in surgical cavities, on the surface of the brain, or in the ventricles of the brain. The choice of technique for transplantation should be determined by the question under investigation, and not the question by the availability of a convenient technique.

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